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A dual role for the FtsK protein in *Escherichia coli* chromosome segregation

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FtsK is a multifunctional protein that acts in *Escherichia coli* cell division and chromosome segregation. Its C-terminal domain is required for XerCD-mediated recombination between *dif* sites that resolve chromosome dimers formed by recombination between sister chromosomes. We report the construction and analysis of a set of strains carrying different Xer recombination sites in place of *dif*, some of which recombine in an FtsK-independent manner. The results show that FtsK-independent Xer recombination does not support chromosome dimer resolution. Furthermore, resolution of dimers by the Cre/*loxP* system also requires FtsK. These findings reveal a second role for FtsK during chromosome dimer resolution in addition to XerCD activation. We propose that FtsK acts to position the *dif* regions, thus allowing a productive synapse between *dif* sites.

INTRODUCTION

The correct segregation of bacterial chromosomes requires that the cell division plane is cleared of DNA before septation is complete (Donachie, 2001; Sawitzke and Austin, 2001). In *Escherichia coli*, chromosome trapping by the division septum may result in inhibition of septum closure if the entrapped DNA is a folded nucleoid (nucleoid occlusion) (Yu and Margolin, 1999; Harry, 2001). Alternatively, if the entrapped DNA links two nucleoids, as in the case of an unresolved chromosome dimer, it may be broken at the septum, leading to chromosome degradation and cell death (Corre *et al.*, 2000; Hendricks *et al.*, 2000).

Chromosome dimers arise by recombination between sister chromosomes. Their resolution is catalysed by two site-specific recombinases of the tyrosine recombinase family, XerC and XerD, which act at the *dif* site, located at the replication terminus of the chromosome (Barre, 2002). Chromosome dimer resolution (CDR) depends on the correct orientation of the sequences flanking *dif*, ~30 kb on each side (Pérals *et al.*, 2000). These sequences must contain polarization signals that condition *dif* recombination at their junction. CDR also depends on FtsK, a large multidomain protein required for cell division (Steiner *et al.*, 1999). The 200 amino acids N-terminal domain of FtsK targets the protein to the division septum and is required for division (Wang and Lutkenhaus, 1998; Yu *et al.*, 1998a). The 600 amino acids of the C-terminal domain of FtsK (FtsKc) display homology with SpoIIIE, which mobilizes the *Bacillus subtilis* chromosome during sporulation, contains an ATP binding motif and is required for CDR (Liu *et al.*, 1998; Yu *et al.*, 1998b; Recchia *et al.*, 1999; Steiner *et al.*, 1999). *In vitro*, a truncated form of FtsK containing a part of the N-terminal domain plus FtsKc, FtsK50C, forms a hexamer that tracks DNA in an ATP-dependent manner and activates *dif* recombination (Aussel *et al.*, 2002). Under the same conditions, FtsKc alone is monomeric and inactive.

Target sites for XerC and XerD are also found on plasmids where they favour resolution of multimers to monomers, hence contributing to plasmid stability (e.g. the *cer* site of ColE1 and the *psi* site of pSC101) (Barre, 2002). The 28–30 bp core sequences of Xer sites are homologous to *dif* and contain the recognition sites for XerC and XerD separated by a 6 bp (*dif* and *psi*) to 8 bp (*cer*) central region where strand exchanges take place. Xer recombination proceeds sequentially, one recombinase exchanging the first pair of strands to create a Holliday junction-containing intermediate that is resolved to products by the action of the second recombinase. Recombination between natural plasmid-borne Xer sites does not depend on FtsK (Recchia *et al.*, 1999), but requires accessory sequences and proteins that restrict recombination to intramolecular events (Barre, 2002).

In this paper, we reveal a dual role for FtsK in CDR using different Xer site core sequences or the *loxP* recombination site

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of bacteriophage P1 to discriminate between the recombination step itself and other FtsK-dependent events.

RESULTS

Replacement of the *dif* site by other Xer core sequences

The different Xer core sequences display various recombination activity when inserted in plasmids and some recombine independently of accessory sequences and of FtsK (Recchia *et al.*, 1999; Table I; in the following, the core sequences will be designed by the name of the site to which they belong). Chosen core sequences were substituted for *dif* on the chromosome (Figure 1; Methods) and the efficiency of CDR in the resulting strains was assessed by microscopic observation (data not shown) and by using a co-culture assay (Pérals *et al.*, 2000; Figure 2). *Psi* and *cer* served, respectively, as positive and negative controls since *psi* had been shown to substitute for *dif* to some extent (Cornet *et al.*, 1994) and *cer* does not recombine in the absence of accessory sequences (Summers and Sherratt, 1988; Leslie and Sherratt, 1995). *cer6* and *cer3* contain the *cer* XerC and XerD arms and differ only in their 6 bp central region (Figure 1). They recombine efficiently when devoid of accessory sequences, both resolving and creating plasmid multimers (Summers, 1989; Guhathakurta *et al.*, 1996). Surprisingly, they behave differently in CDR: *cer3* resolves about two-thirds of the chromosome dimers formed whereas *cer6* does not improve the viability defect due to the deletion of *dif* (Figure 2; Table I). This shows that a core sequence containing the XerC and XerD binding sites of *cer* can resolve chromosome dimers and suggests that the central region is an important determinant of this activity.

Resolution of chromosome dimers by Xer core sequences depends on FtsK

Recombination at *dif* (whether plasmid or chromosome-borne) depends on FtsK while recombination at *cer6* does not (Recchia *et al.*, 1999; Figure 3). Figure 3 shows that inactivation of FtsKc has no effect on recombination at plasmid-borne *cer3*. However, inactivation of FtsKc impairs CDR in all strains, including the one carrying *cer3* in place of *dif* (Figure 2; Table I). Thus, CDR by *psi* and *cer3* depends on FtsK whereas recombination at *psi* and *cer3* does not.

Table I. Recombination activity of different Xer core sequences

	Recombination on plasmids		CDR	
	fts wt	*ftsK*::Cm	*ftsK* wt	*ftsK*::Cm
dif	+	–	+++	–
psi	+/–	+/–	++	–
cer	–	–	–	–
cer3	++	++	++	–
cer6	+++	+++	–	–

This table summarizes the respective *in vivo* recombination activity of the different Xer sequences core in *ftsK* wt and *ftsK*::Cm strains. Recombination on plasmids was assayed using reporter plasmids carrying directly repeated core sequences. CDR was measured using the co-culture assay. +++, level of the most active site (*dif* for CDR and *cer6* for recombination on plasmid); ++, 50–70% of the +++ activity; +, 10–50%; +/–, <10%; –, undetectable activity. For recombination on plasmid, results are redrawn from references cited in the text and from Figure 3.

```
wt  AGACATG ATTTAACATAA  TATACA  TTATGCGCACC AATATAACCAAG
dif AGATCTG ATTTAACATAA  TATACA  TTATGCGCACC AATATAACGCGT
psi AGATCTG GTTTAACATAA  TGGATC  TTGCGCGCACC AATATAACGCGT
cer AGATCTG ATTTACCATAA TCCCTTAA TTGTACGCACC AATATAACGCGT
cer3 AGATCTG ATTTACCATAA  CTCCCG  TTGTACGCACC AATATAACGCGT
cer6 AGATCTG ATTTACCATAA  CATCCC  TTGTACGCACC AATATAACGCGT
      BglII      XerD       CR       XerC          MluI
```

Fig. 1. Xer core sequences used to replace *dif*. wt, the wild-type *dif* sequence; *dif*, reinsertion of *dif* as an *Mlu*I–*Bgl*II linker. The difference core sequences are shown together with the XerC and XerD binding sites, the central region (CR) and modifications of the sequence to create *Bgl*II and *Mlu*I sites. Differences between *cer3* and *cer6* are shown in bold.

Resolution of dimers by the Cre/*loxP* system depends on FtsK while Cre/*loxP* recombination does not

We next asked whether the Cre/*loxP* system, which can substitute for XerCD/*dif* (Leslie and Sherratt, 1995), resolves dimers in the absence of FtsKc. Two directly repeated *loxP* sites separated by a resistance determinant to kanamycin (Kn) were inserted in place of *dif* (see Methods). Transformation of the resulting strain, FC149, with the Cre-expressing plasmid, pFX71, yielded a CDR^+ phenotype (Figure 4). In contrast, strains carrying *loxP* sites inserted at position *zdc310* or *zdd370*, located 37 kb to the left and 23 kb to the right of the *dif* position, respectively, were CDR^- (data not shown), showing that CDR by the Cre/*loxP* system is subject to the same positional control as CDR by XerCD/*dif*. Introduction of the *ftsK*::Cm allele into FC149 resulted in extensive formation of filaments and chains characteristic of *ftsKc* strains that were not suppressed by the presence of Cre (Figure 4). The absence of suppression was confirmed using co-culture experiments (data not shown). Thus, CDR by the Cre/*loxP* system depends on FtsK.

In each strain, transformation with pFX71 led to a rapid loss of Kn resistance (<5% of the transformants gave colonies on Kn-containing plates), showing that the *loxP* sites recombine at high frequency independently of FtsK.

The viability defect due to inactivation of *dif* and FtsKc are non-additive and depend on RecA

Because dimers form by homologous recombination, the deleterious effects of XerCD/*dif* inactivation on division and viability depends on RecA (Pérals *et al.*, 2000). Similarly, removal of RecA function in an *ftsK*::Cm mutant suppresses the filamentous phenotype (Recchia *et al.*, 1999). Co-culture experiments show that the viability defect due to the *ftsK*::Cm mutation is also suppressed by inactivation of RecA (Figure 5; data not shown). These observations suggest that FtsK has no discernible role in chromosome segregation apart from CDR. Consistent with this hypothesis, viability is reduced to the same extent in *xerC*, *ftsKc* and *xerC ftsKc* mutants (Figure 5).

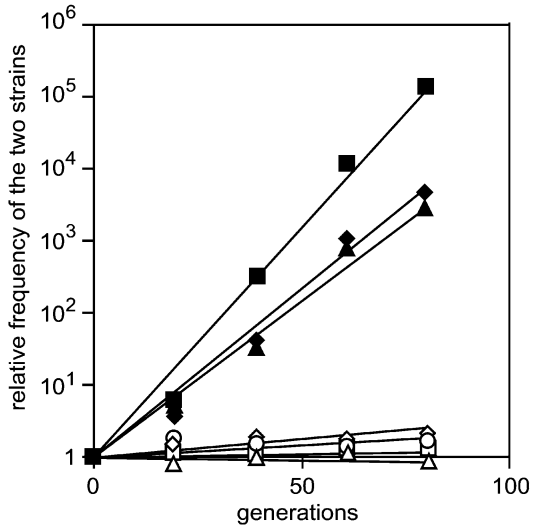

Fig. 2. Growth advantage of strains carrying the different Xer core sequences in place of *dif* over a Δ(*dif*) strain. Strains carrying the *cer3* (closed diamonds), *psi* (closed triangles), *cer6* (open circles) or *cer* (open diamonds) core sequences in place of *dif* were mixed with strain LN2772 [Δ(*dif*)58::Tc] and grown in serial culture. The relative frequency of the two strains is plotted as the Tc^S/Tc^R ratio. Closed squares, *dif* reinserted as an *Mlu*I–*Bgl*II linker; open squares, co-culture of strains LN2772 and FC223 (*xerC*::Cm); open triangles, co-culture of LN4263 (LN2772 *ftsK*::Cm) and LN4262 [Δ(*dif*)::*cer3 ftsK*::Cm].

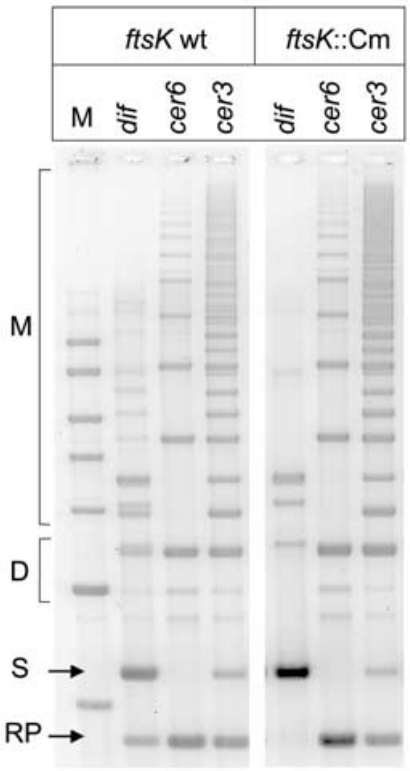

Fig. 3. Recombination between plasmid-borne core sequences. Strains FX55 (*ftsK* wt) and FX60 (FX55 *ftsK*::Cm) were transformed with plasmids containing direct repetitions of the core sequences: pBH220 (*dif*), pBH222 (*cer6*) or pBH223 (*cer3*). Plasmid DNA was recovered after overnight growth and analysed by agarose gel electrophoresis. Positions of the substrate (S), the recombination product containing one core sequence (RP) dimers of S and RP (D), and higher multimers (M) formed by Xer recombination are indicated on the left. Note the disappearance of the forms corresponding to plasmids carrying the initial repetition of *cer6* due to a higher rate of recombination at *cer6* than at *cer3* and *dif*.

DISCUSSION

In this report, we have reinvestigated the functional replacement of *dif* by other Xer site core sequences in light of the recently described FtsK-dependent pathway of Xer recombination. The results obtained have implications for the role of FtsK in CDR and chromosome segregation, and for the mechanism of FtsK-driven Xer recombination.

Replacement of *dif* by *psi*, *cer3* or *loxP* leads to uncoupling of recombination and CDR. These sites recombine but do not resolve dimers in the absence of FtsKc. The role of FtsK during CDR is therefore not restricted to the activation of the recombination event *per se*. This explains, and is reinforced by, two observations: (i) expression of the C-terminal domain of FtsK in *ftsKc* strains activates recombination between *dif* sites but does not restore the CDR^+ phenotype (Barre *et al.*, 2000; Pérals *et al.*, 2001); (ii) overexpression of FtsK reactivates recombination between displaced *dif* sites without activating their CDR activity (Pérals *et al.*, 2001). Since CDR, by the Cre/*loxP* system, is submitted to the same positional control as CDR by XerCD/*dif*, we propose that FtsK actively positions *dif* (or its substitutes) by tracking DNA specifically towards *dif*. This may allow synapsis of the *dif* sites in or near the septum and FtsK-dependent activation of XerCD catalysis. FtsK may thus be a major actor of the positional control exerted on *dif* activity. It is tempting to propose that FtsK uses the determinants of the functional polarization detected in the *dif* region to orientate its tracking activity (Pérals *et al.*, 2000). This idea has been reinforced recently by a study of the distribution of chromosome lesions that result from FtsKc inactivation (Corre and Louarn, 2002).

Xer recombination follows different pathways depending on the presence of accessory sequences and proteins, and FtsK (Barre *et al.*, 2002; Table I). The central region of the Xer sites, which displays no consensus, is a key determinant of the FtsK-dependent pathway since *cer3* and *cer6* display opposite behaviours in CDR. Several set of data, obtained on the Xer system and other tyrosine recombinase systems, indicate that this region is an important determinant of the conformation of the recombinase–core sequence complexes (Azaro and Landy, 1997; Gopaul *et al.*, 1998; Arciszewska *et al.*, 2000; Lee and Sadowski, 2001). Recent results suggest that during *dif* recombination, XerD exchanges the first pair of strands in an FtsK-dependent manner (Aussel *et al.*, 2002), whereas in FtsK-independent pathways XerC acts first (Barre *et al.*, 2002). The sequence of the central region may therefore allow adoption of the conformation appropriate for XerD to exchange the first pair of strands during FtsK-driven recombination.

Speculation

Inactivation of FtsKc results in the same level of defect in viability as inactivation of XerCD/*dif* and is likewise suppressed by

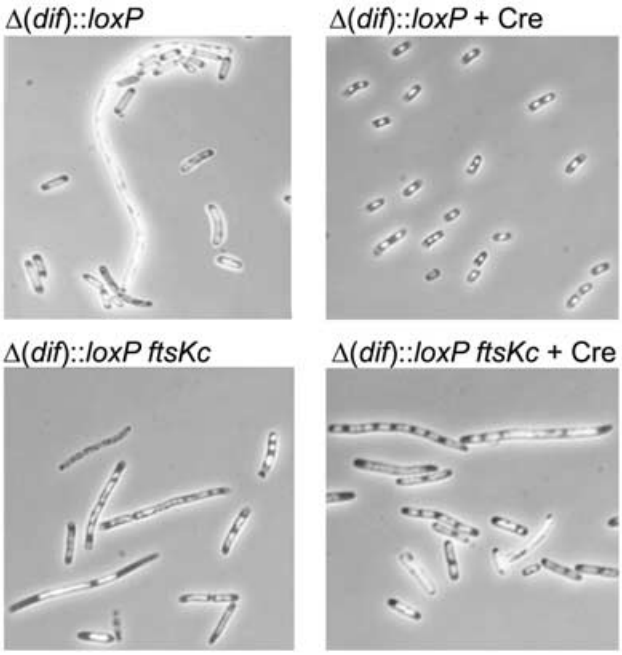

Fig. 4. Phase-contrast fluorescence micrographs of strains carrying the *loxP* site in place of *dif* (DAPI staining). Top left, FC149 [LN2666 Δ(*dif*)::Tc::*loxP*–Kn–*loxP*]; top right, FC149/pFX71; bottom left, FC407 (FC149 *ftsK*::Cm); bottom right, FC407/pFX71.

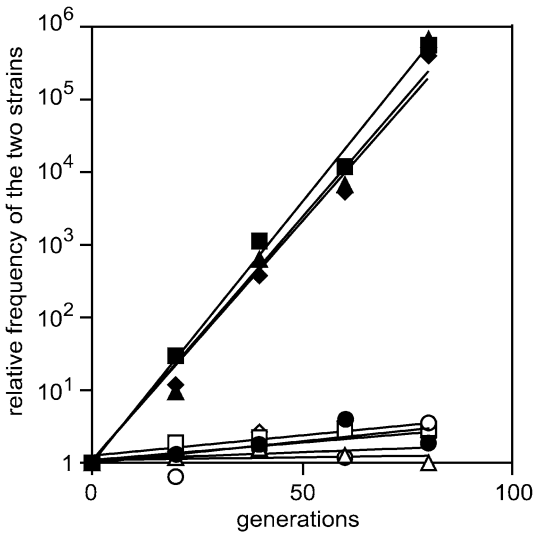

Fig. 5. Viability defect due to inactivation of *dif* and FtsKc. Strains LN2666 (wt), FC126 [Δ(*dif*)2600::Gm], FC405 (*ftsK*::Cm) and FC318 [Δ(*dif*)2600::Gm *ftsK*::Cm] were mixed by pairs and grown in serial culture. Closed symbols, LN2666 versus each mutant strain. The relative frequency of LN2666 is plotted. Diamonds, LN2666 versus FC126; squares, LN2666 versus FC405; triangles, LN2666 versus FC318; circles, FC232 (LN2666 *recA56*) versus FC420 [Δ(*dif*) *ftsK*::Cm *recA56*]. Open symbols: mutant strain versus mutant strain. The frequency of the first strain cited is plotted. Diamonds, FC126 versus FC318; squares, FC126 versus FC405; circles, FC405 versus FC318; triangles (control), FC126 versus LN3079 [Δ(*dif*)2600::Tc].

inactivation of RecA. Thus, the only role of FtsK in chromosome segregation may be in CDR. However, the DNA tracking activity of FtsK and its homology with SpoIIIE suggest a more general role for FtsK in clearing the division plane of DNA. Our data may be reconciled with this idea assuming that the vast majority of DNA entrapped at the septum belongs to chromosome dimers. This may vary with growth conditions and genetic background. For instance, the co-lethality observed for *mukB* and *ftsKc* mutations (Yu *et al.*, 1998b) may be due to a higher proportion of entrapped DNA in the absence of MukB. This hypothesis is consistent with the less-condensed nucleoid and the 'guillotine' effect observed in *mukB* mutants (Niki *et al.*, 1991; Weitao *et al.*, 2000; Sawitzke and Austin, 2001). Inactivation of PriA is also co-lethal with inactivation of FtsKc but not with inactivation of XerCD/*dif* (McCool and Sandler, 2001). This observation is the first reported example of FtsKc mutations having effects distinct from those of XerCD/*dif* mutations. We propose that FtsK acts to segregate any sequences entrapped at the septum. This activity mostly concerns dimers in wild-type cells but becomes essential for segregation of monomers when the frequency of entrapped DNA rises due to inactivation of chromosome condensation, replication or segregation functions.

METHODS

Strains and plasmids. Strains were derived from LN2666 (W1485 *leu thyA deoB* or *C supE rpsL*). LN2772 is LN2666 Δ(*dif*)58::Tc. LN3079 is LN2666 Δ(*dif*)2600::Tc (Cornet *et al.*, 1994, 1996). The *recA56* allele was co-transferred with *srl*::Tn*10* from strain JC10240 (Hfr PO45 *recA56 srl*::Tn*10 thr300 ilv318 rpl300*). The *ftsK*1 (*ftsK*::Cm) allele was introduced into strains by P1-mediated transduction. Plasmid pFX71 (pBAD18-Cre) was a gift from F.X. Barre (Department of Molecular Genetics, Oxford, UK).

Xer core sequences were introduced on the chromosome via derivatives of plasmid pFC214, which consists of pLN135 (Cornet *et al.*, 1996) carrying the *Eco*RV 1715 bp chromosomal *dif* fragment modified by site-directed mutagenesis to introduce the *Mlu*I and *Bgl*II restriction sites (Figure 1). Xer core sequences were constructed by annealing of relevant oligonucleotides and cloned into pFC214 in place of *dif* as *Mlu*I–*Bgl*II linkers. Resulting plasmids were used to replace the Tc^R determinant tagging the Δ(*dif*)58 deletion in LN2772 by the different core sequences following the general procedure described for use of this family of plasmids (Cornet *et al.*, 1994, 1996).

The *loxP*–Kn–*loxP* construct was introduced into chromosome-borne Tc fragments via plasmid pFC224 in several steps. A Kn determinant was first cloned into the *Pst*I site of pN40 (pMTL23–*loxP*) (Leslie and Sherratt, 1995). The resulting *Stu*I–*Mlu*I fragment carrying *loxP* and Kn was cloned into the *Stu*I–*Eco*RI sites of pN40, creating the *loxP*–Kn–*loxP* cassette, which was inserted as a *Stu*I–*Eco*RV fragment into the *Eco*RV site of pFC68 (Cornet *et al.*, 1996), creating the Tc::*loxP*–Kn–*loxP* construct.

General procedures. Experiments were carried out in L broth and involved standard procedures (Miller, 1992). Plasmid DNA was purified using a GenElute plasmid miniprep kit from Sigma. The Altered Sites *in vitro* Mutagenesis system from Promega was used for site-directed mutagenesis. For microscopic observations, bacteria were grown to an OD_{600} of 1 in L broth, incubated for 30 min at 37°C in L broth containing 5 μg/ml of 4,6-diamino-2-phenylindol (DAPI), recovered in M9 medium and observed with a Leica DMRB microscope.

Plasmid recombination assay. Plasmids containing a direct repetition of *cer3*, *cer6* or *dif* (pBH220, pBH222 and pBH223, respectively; from Bernard Hallet, Université Catholique de Louvain, Louvain la Neuve, Belgium) were introduced by transformation into strains FX55 (*ftsK* wt) and FX60 (*ftsK*::Cm) (Aussel *et al.*, 2002). Transformants were recovered as pools of 20 colonies, plasmid DNA was extracted and analysed by electrophoresis in a TAE 1% agarose gel.

Co-culture assay. The co-culture assay measured the selective advantage of a strain compared with a Δ(*dif*) strain (Pérals *et al.*, 2000). Briefly, a 1:1 mixture of the two strains was grown in serial culture in L broth at 37°C and their relative frequencies were determined by plating every 20 generations. The rate at which a wild-type strain takes over an isogenic CDR$^-$ strain corresponds to ~14% of abortive division due to unresolved chromosome dimers in the CDR$^-$ strain.

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